II. "Experimental Researches on Vegetable Assimilation and Respiration.—No. II. On the Paths of Gaseous Exchange between Aerial Leaves and the Atmosphere." By F. F. BLACKMAN, B.Sc., B.A., St. John's College, Demonstrator of Botany in the University of Cambridge. Communicated by FRANCIS DARWIN, F.R.S. Received November 15, 1894.

(Abstract.)

On the question of the path by which carbonic acid passes out of the leaf in respiration and into it in assimilation, whether this takes place by the stomatal openings or through the continuous surface of the cuticle, all possible extreme and intermediate views have been expressed in recent text-books of botany. On account of the smallness of the quantities of gas involved, practically no attempt has hitherto been made to determine this question by direct estimation. The existing experimental evidence is all of an indirect nature, and tends rather to support the view that the exchange is a cuticular phenomenon.

An ingenious synthesis of Graham's observations on the comparative readiness with which CO_2 osmoses through thin films of caoutchouc, with observations by Frémy and others on the similarity between cuticle and caoutchouc in chemical composition, first led Barthélemy (1868) to put forward the view that the cuticle was specially adapted for transmitting CO_2 from the external air to the assimilating cells beneath it. This view he supported by experiments on the artificial osmosis of gases through leaves. About the same time Boussingault performed experiments that seemed to definitely show that in assimilation, the CO_2 taken up by the leaf entered it through the upper surface, devoid of stomata, to which the assimilating cells are adjacent, rather than through the more distant stomatal openings. These experiments have hitherto been generally accepted, but I shall show later that the conclusions drawn from them are entirely fallacious. In support of the view that stomata form the paths of gaseous exchange, besides scattered inductions by various workers, we have the conclusion arrived at in 1888 by Mangin, from diffusion experiments on isolated cuticle, that this diffusion is insufficient to account for the whole gaseous exchange of the leaf.

By the aid of the apparatus described in a previous paper, the author has been able successfully to attack the problem directly by estimating the amounts of CO_2 given out or taken in by the two surfaces of the same leaf, under the same conditions. For this pur-

pose, shallow capsules 10 sq. cm. in area, consisting of a glass plate with a metal rim, through which tubes for the circulation of the air current pass, are employed. Two of them are affixed to a leaf on opposite sides of the same area in air-tight union by means of soft wax. Then, in the way described previously, two continuous currents of air can be kept up over the two surfaces, and the CO_2 produced or taken in, during a given time, by each of them be determined.

Numerous experiments on the respiration of a variety of leaves, thick and thin, with the stomata all on the one side or with stomata variously distributed on the two sides, agree in showing that the stomata are the site of the exhalation of this gas. When no stomata are present on the upper surface of a leaf, then practically no CO_2 is exhaled from that surface, at least, not more than falls within the working error of the apparatus under these conditions, while more than thirty times as much may be given off from the lower stomatiferous surface. When stomata occur on both surfaces, the relative amounts of CO_2 exhaled closely follow the ratios of the numbers of stomata.

A few examples in illustration of this may be given.

	A.	B.		C.
Ampelopsis hederacea	$\frac{0}{S}$	$\frac{0 \cdot 003}{0 \cdot 100}$	c.c. CO_2 per hour per 10 sq. cm.....	$\frac{3}{100}$
Alisma Plantago....	$\frac{135}{100}$	$\frac{0 \cdot 030}{0 \cdot 025}$	,, ,, ,, 10 sq. cm.....	$\frac{120}{100}$
Iris germanica......	$\frac{100}{100}$	$\frac{0 \cdot 029}{0 \cdot 026}$	,, ,, ,, 10 sq. cm.....	$\frac{110}{100}$
Ricinus communis...	$\frac{100}{250}$	$\frac{0 \cdot 015}{0 \cdot 037}$	,, ,, ,, 10 sq. cm.....	$\frac{100}{260}$

Column A gives the proportionate numbers of stomata on the two surfaces; B the amounts of CO_2 exhaled by these; C the ratios of these amounts. Each experiment lasted about fifteen hours.

Experiments on the absorption of CO_2 during assimilation showed the same close relation to the distribution of stomata. For these experiments a constant fixed amount of CO_2 must be introduced into the air stream supplied to the leaf, which makes them much more complicated.

As hitherto carried out, direct sunshine, continuous for several hours, has been essential for the success of these experiments. They are, consequently, but few in number, though perfectly clear in their interpretation.

In an experiment on the leaf of *Ampelopsis hederacea*, with no stomata on the upper surface, air containing 1·6 per cent. CO_2 was supplied to both surfaces at the rate of 22 c.c. for every fifteen minutes; of the 0·37 c.c. of CO_2 thus entering the capsule on the lower surface in this time, 0·14 c.c. was absorbed, while none at all was absorbed by the upper surface. With a leaf of *Alisma*, on the contrary, the whole of the CO_2—0·15 c.c.—supplied in fifteen minutes to the upper surface was absorbed, and 0·11 c.c. of that supplied to the lower. In this leaf the stomata are in the ratio of 135 above to 100 below, to which ratio the absorption numbers closely correspond. A very simple experiment will show that stomata are practically the sole path of entry of CO_2 for assimilation. If part of the lower stomatic surface of any leaf with no stomata on its upper surface (*Sparrmannia* gives very clear results) be coated with wax so as to mechanically block the stomata, no starch can be formed in that area, while the adjacent areas become rich in starch. I performed this experiment in 1893, and showed it to some botanists; recently it has been published by Stahl.*

As stated previously, the theory of "cuticular exchange" has hitherto found its strongest support in the experiments of Boussingault, in which, under similar conditions, leaves of *Nerium Oleander* assimilated less when the upper astomatiferous surface had been coated with an unguent than when the lower stomatiferous surface had been so coated. From this he drew the obvious conclusion that the CO_2 of assimilation normally passes into the leaf through the cuticle of the upper surface. Exposure of the interesting experimental fallacy here concealed, however, quite reverses the interpretation of these experiments. Boussingault experimented with leaves in an atmosphere containing 30 per cent. CO_2. Now the optimum percentage of CO_2 for assimilation is very low for this leaf, and the real interpretation of the result is that the diminished decomposition of CO_2 in the leaf with open stomata is due to its obtaining not *less* CO_2 but *more* CO_2. In fact, there penetrates into it so much CO_2 that its assimilatory activity is lessened, and falls below that of the other leaf into which, owing to the blocking of the stomata, the CO_2 only diffuses very slowly, and cannot exceed the optimal strength. This view has been conclusively proved by a series of experiments in different strengths of CO_2. In a small percentage the leaf with its stomata open decomposes more CO_2 than the leaf with its stomata blocked—a result just the reverse of Boussingault's.

Further evidence on the possible paths of gaseous exchange has been obtained by investigating the degree to which diffusion of CO_2 can be artificially produced through the living leaf. Strong mixtures of CO_2 are led continuously across one surface of a leaf, and the

* 'Botanische Zeitung,' July, 1894.

amount which diffuses through is estimated. On supplying 31 per cent. CO_2 to the lower surface of a leaf of *Nerium* only 0·035 per cent. appears by diffusion in a slow current of air kept continually passing over the upper surface of the leaf.

Other experiments on the respiration of injected leaves also support the view that the stomatal openings, in spite of their minuteness, offer a very much easier path from the atmosphere to the interior of the leaf than does the cuticle.

Conclusions.—1. Under normal conditions practically the sole pathway for CO_2 into or out of the leaf is by the stomata. Since oxygen diffuses more readily than CO_2 through fine openings, the same probably holds for oxygen and the whole of the gas exchange.

2. Under abnormal conditions, when the stomata or intercellular spaces are blocked and the surrounding tension of CO_2 is great enough, passage of CO_2 by osmosis through the cuticle may take place.

3. That such closure of stomata as is held to take place in darkness does not prevent the distribution of gas exchange closely agreeing with that of the stomata.

4. That the exhalation of CO_2 in bright light by a leafy shoot in Garreau's well-known experiment is not the expression of any physiological truth for the leaf, but only due to the imperfections of the conditions; to the presence of immature parts, or of tissues not sufficiently green or not fully illuminated. Mature isolated green leaves fully illuminated assimilate the whole of their respiratory CO_2 and allow none to escape from them.

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